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Deficient Vesicular Storage: A Common Theme in Catecholaminergic Neurodegeneration

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Abstract

Several neurodegenerative diseases involve loss of catecholamine neurons—Parkinson's disease (PD) is a prototypical example. Catecholamine neurons are rare in the nervous system, and why they are lost has been mysterious. Accumulating evidence supports the concept of “autotoxicity”—inherent cytotoxicity caused by catecholamine metabolites. Since vesicular sequestration limits the buildup of toxic products of enzymatic and spontaneous oxidation of catecholamines, a vesicular storage defect could play a pathogenic role in the death of catecholaminergic neurons in a variety of neurodegenerative diseases. In putamen, deficient vesicular storage is revealed in vivo by accelerated loss of ¹⁸F-DOPA-derived radioactivity and post-mortem by decreased tissue dopamine (DA):DOPA ratios; in myocardium in vivo by accelerated loss of ¹⁸F-dopamine-derived radioactivity and post-mortem by increased 3,4-dihydroxyphenylglycol:norepinephrine (DHPG:NE) ratios; and in sympathetic noradrenergic nerves overall in vivo by increased plasma F-dihydroxyphenylacetic acid (F-DOPAC):DHPG ratios. We retrospectively analyzed data from 20 conditions with decreased or intact catecholaminergic innervation, involving different etiologies, pathogenetic mechanisms, and lesion locations. All conditions involving parkinsonism had accelerated loss of putamen ¹⁸F-DOPA-derived radioactivity; in those with post-mortem data

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there were also decreased putamen DA:DOPA ratios. All conditions involving cardiac sympathetic denervation had accelerated loss of myocardial ^{18}F -dopamine-derived radioactivity; in those with post-mortem data there were increased myocardial DHPG:NE ratios. All conditions involving localized loss of catecholaminergic innervation had evidence of decreased vesicular storage specifically in the denervated regions. Thus, across neurodegenerative diseases, loss of catecholaminergic neurons seems to be associated with decreased vesicular storage in the residual neurons.

Graphical Abstract

Keywords

Catecholamine; Dopamine; Norepinephrine; Parkinson's disease; Neurodegeneration

The movement disorder in Parkinson's disease (PD) is well known to be associated with—and largely result from—loss of substantia nigra dopaminergic neurons that project to the striatum. Profound striatal dopamine (DA) deficiency, especially in the putamen, accompanies this loss [1]. PD is also associated with cardiac sympathetic denervation [2–4] and severe depletion of myocardial norepinephrine (NE) [5].

It is widely presumed that catecholamine depletion in PD directly reflects loss of nigrostriatal and sympathetic neurons; however, both in the putamen and myocardium, the extent of catecholamine depletion in PD is greater than can be accounted for solely by the loss of nigral dopaminergic or post-ganglionic noradrenergic neurons [1, 3, 5–9].

The difference might be explained by decreased efficiency of vesicular storage of catecholamines in the residual neurons. In PD, indices of catecholaminergic denervation and decreased vesicular storage are linked, both in putamen dopaminergic terminals [8, 10, 11] and in sympathetic nerves [5, 12, 13].

According to the concept of catecholamine “autotoxicity,” this linkage is causal. As illustrated in Figure 1, cytoplasmic catecholamines are toxic, via oxidative injury and protein modifications mediated by products of spontaneous and enzyme-catalyzed oxidation. Since vesicular sequestration is a major determinant of the cytoplasmic concentrations of

intra-neuronal catecholamines, a vesicular storage defect could play a pathogenetic role in the death of catecholaminergic neurons.

The parkinsonian form of multiple system atrophy (MSA-P) clinically resembles PD with orthostatic hypotension (PD+OH) [14, 15]. Both are forms of synucleinopathy, but PD is characterized by intra-neuronal alpha-synuclein deposition [16], while MSA is characterized by glial cytoplasmic inclusions containing alpha-synuclein, mainly in oligodendrocytes [17, 18]. A recent post-mortem study indicated that in MSA, as in PD, putamen DA depletion is accompanied by neurochemical evidence for a vesicular storage defect [12], and a recent in vivo study indicated that a plasma biomarker of a vesicular storage defect distinguishes MSA-P from PD+OH [12]. Moreover, although most MSA patients have intact cardiac noradrenergic innervation [19], a minority have denervation [20–22], and a recently reported case of MSA-P with cardiac sympathetic denervation had post-mortem neurochemical evidence for a vesicular storage defect both in the putamen and myocardium [23]. Pure autonomic failure (PAF), a rare but scientifically important Lewy body disease [24, 25] manifesting with neurogenic orthostatic hypotension, also features deficient vesicular storage in the residual noradrenergic nerves [13].

The findings in PD+OH, MSA-P, and PAF prompted the present examination of whether, as a general phenomenon in neurodegenerative diseases, catecholaminergic denervation is associated with decreased vesicular storage in the residual neurons. We conducted a retrospective analysis of data from patients with diseases involving different etiologies, likely pathogenetic mechanisms, and lesion locations. Using the same approach of in vivo neuroimaging and neurochemistry and confirmatory post-mortem neurochemistry when available, we assessed the status of catecholaminergic innervation in the brain or periphery in these diagnostic groups. We included data from several rare but informative conditions such as PARK1, PARK4, LRRK2/PD (PARK8), Gaucher/PD, PAF, MSA with and without cardiac denervation, progressive supranuclear palsy (PSP), amyloidosis with neurogenic orthostatic hypotension, familial dysautonomia (FD), and autoimmune autonomic ganglionopathy (AAG).

METHODS TO ASSESS VESICULAR STORAGE IN CATECHOLAMINERGIC NEURONS

Several in vivo and post-mortem approaches have been used for assessing vesicular storage in putamen dopaminergic and sympathetic noradrenergic neurons. The following summarizes the ones we used; detailed descriptions are found in an Appendix.

Putamen Dopaminergic Innervation and Vesicular Storage

In vivo—After i.v. administration of ^{18}F -DOPA, peak putamen ^{18}F -DOPA-derived radioactivity is attained at about 30 minutes after i.v. injection of the tracer. Subsequently, putamen radioactivity normally declines slowly over a few hours (Figure 2A). The rate of decline can be quantified from the fractional decrease in radioactivity between the mid-point of the 15' scan beginning at 30' and the mid-point of the 15'-minute scan ending at 120' (Fractional Loss 30'–120'). Denervation alone would be expected to shift downward the

curve relating putamen radioactivity vs. time, without a change in the slope (blue arrow in Figure 2B). A vesicular storage defect in the residual terminals would increase the rate of decline in ^{18}F -DOPA-derived radioactivity (Figure 2C).

All the patients were studied at the NIH Clinical Center. In virtually all patients the same ^{18}F -DOPA scanning protocol was used. Dynamic scanning was done in a conventional scanner for 30 minutes followed by a 15-minute static scan; the patient was transferred to a high resolution research tomograph for a 15-minute static scan; and the patient was then transferred back to the conventional scanner for a final 15-minute static scan timed to end at about 120 minutes from the time of initiation of the 3-minute injection of ^{18}F -DOPA injection. For analyzing the images, regions of interest placed on the MRI of each patient. No carbidopa pre-treatment was used, as we found in preliminary studies that this was not necessary. The amount of F-DOPA administered, 7 mCi, was also less than in most other studies, again because in preliminary studies higher doses (and greater radioactivity exposures) were unnecessary. In a supplementary Table we provide the mean values and standard errors for PUT and OCC ^{18}F -DOPA-derived radioactivity in normal control, PD, and MSA groups for each scan.

The fractional loss of putamen (PUT) radioactivity was measured between the midpoint of the 15-minute static image beginning at 30' (mean about 38') and the midpoint of the last 15-minute static image ending at 120' (mean about 113'). The fractional loss was called Fractional Loss 30'–120'.

Post-mortem—By reference to Figure 1, since the putamen tissue content of DA is for all practical purpose in the vesicles, while DA is produced in the cytoplasm from DOPA, the immediate product of the rate-limiting enzyme in DA synthesis (tyrosine hydroxylase, TH), a vesicular storage defect in putamen dopaminergic terminals would be expected to result in a proportionately greater decrease in the tissue content of DA than of DOPA and therefore in a decreased DA:DOPA ratio [8]. An association of putamen dopaminergic denervation with a vesicular storage defect in the residual terminals would be reflected by an inverse relationship between putamen DA:DOPA ratios and DA contents across diagnostic groups.

Sympathetic Noradrenergic Innervation and Vesicular Storage

In vivo

Cardiac noradrenergic denervation: If a vesicular storage defect characterized degenerating cardiac sympathetic neurons, then after i.v. injection of the sympathetic neuroimaging agent, ^{18}F -DA, patients with cardiac sympathetic denervation would have accelerated loss of the radioactivity, in a manner analogous to that for ^{18}F -DOPA in the putamen. By reference to Figure 2D, peak ^{18}F -DA-derived radioactivity normally is attained at about 8 minutes after initiation of the 3-minute i.v. infusion of the tracer. The radioactivity at 8 minutes (8' Radioactivity) can be used as a measure of neuronal uptake and thereby of innervation. Subsequently the concentration of ^{18}F -DA-derived radioactivity decreases slowly. The slope of the mono-exponential decline between 8' and 25', referred to as $k_{8'-25'}$, can be taken as a measure of this loss. Because of decreased neuronal uptake, denervation would be expected to shift downward the curve relating the log of

interventricular septal myocardial ^{18}F -DA-derived radioactivity vs. time, without a change in the slope of the mono-exponential decline. That is, $8'$ Radioactivity would be decreased, without a change in $k8'-25'$. Denervation combined with decreased vesicular uptake would be expected to both decrease $8'$ Radioactivity and increase $k8'-25'$. An association between a vesicular storage defect and cardiac sympathetic denervation would be reflected by an inverse relationship between $k8'-25'$ and mean $8'$ Radioactivity across patient groups.

Generalized noradrenergic denervation: After neuronal uptake of F-DA, cytoplasmic F-DA is mainly taken up into the vesicles, but a minority is deaminated to form F-DOPAC. If there were generalized sympathetic denervation without a vesicular storage defect, then peak arterial plasma F-DOPAC would be decreased [26], but if there were a defect in vesicular storage without denervation, then peak arterial plasma F-DOPAC would be increased. That is, the effect of one abnormality could offset the effect of the other abnormality. To detect a vesicular storage defect in the setting of denervation, one can adjust F-DOPAC for plasma 3,4-dihydroxyphenylglycol (DHPG), which reflects turnover of NE stores in sympathetic nerves and therefore overall sympathetic innervation [12]. We used peak arterial F-DOPAC:DHPG as a biomarker of decreased vesicular sequestration in the sympathetic nervous system as whole.

If generalized loss of sympathetic noradrenergic nerves were associated with a vesicular storage defect, regardless of the specific disease, then across conditions peak F-DOPAC:DHPG in arterial plasma would be related inversely to arterial plasma DHPG. In PD without orthostatic hypotension (PD No OH), noradrenergic denervation seems to be localized to the heart, whereas PD+OH and PAF involve generalized noradrenergic denervation. We therefore predicted normal peak arterial plasma F-DOPAC:DHPG in PD No OH.

Post-mortem—If cardiac sympathetic denervation were associated with a vesicular storage defect, then across patient groups with different diseases, in the groups with myocardial NE depletion the pattern of post-mortem catechols in the left ventricular myocardium would indicate a vesicular sequestration-to-oxidative deamination shift in residual noradrenergic nerves. An elevated ratio of DHPG:NE provides an index of this shift [8].

CATECHOLAMINERGIC DENERVATION IS ASSOCIATED WITH DECREASED VESICULAR STORAGE ACROSS COMPARED GROUPS

We reviewed In vivo or post-mortem data across a total of 20 different diagnostic groups characterized in terms of intact or decreased catecholaminergic innervation in the brain or periphery. This section and Table 1 provide overviews; detailed descriptions of each group are in an Appendix.

Putamen in vivo ^{18}F -DOPA data were reviewed from a total of 142 subjects and postmortem DA:DOPA data from a total of 53 subjects. Myocardial in vivo ^{18}F -DA data were reviewed from a total of 271 subjects, peak arterial plasma F-DOPAC:DHPG 86 subjects, and post-mortem DHPG:NE data 64 subjects.

The patient groups included six with types of PD—the PARK1, PARK4, and PARK 8 forms of familial PD, PD in the setting of Gaucher disease (Gaucher/PD), PD with orthostatic hypotension (PD+OH), and PD without OH (PD No OH). In post-mortem studies, PD+OH and PD No OH were combined (PD (Combined)).

There were three groups with multiple system atrophy (MSA)—the parkinsonian form (MSA-P), the cerebellar form (MSA-C), and MSA with autopsy proven cardiac sympathetic denervation and no intra-neuronal deposition of alpha-synuclein (MSA+D). In post-mortem studies the MSA groups were combined (MSA (Combined)).

A group was included with progressive supranuclear palsy (PSP). There were 2 groups with neurogenic OH without signs of central neurodegeneration—pure autonomic failure (PAF) and amyloidosis (AMYL). Other comparison groups included familial dysautonomia (FD, also known as type III hereditary sensory and autonomic neuropathy), autoimmune autonomic ganglionopathy (AAG), postural tachycardia syndrome (POTS), status-post bilateral endoscopic thoracic sympathectomies (SNS-x), referred patients without clinical evidence of central neurodegeneration or autonomic failure (Control), and healthy volunteers. Among the latter, subgroups underwent ^{18}F -DA scanning in the setting of pharmacologic manipulations altering sympathetic noradrenergic outflows.

As described in detail below, catecholaminergic denervation and vesicular storage are linked in putamen dopaminergic terminals and in sites of sympathetic noradrenergic innervation, based on in vivo neuroimaging and neurochemical data supplemented by post-mortem neurochemical data.

In Vivo Putamen Dopaminergic Innervation

Vesicular Storage—Of four conditions involving parkinsonism, all four had accelerated loss of the radioactivity, in contrast with normal rates of loss of the radioactivity in all of six control groups (Figures 3 and 4). The PD+OH and PD No OH groups had similarly high fractional losses of radioactivity. The MSA-P group also had high mean Fractional Loss 30'–120', whereas the MSA-C group had closer to normal values. Nevertheless, mean Fractional Loss 30'–120' in MSA-C (0.20 ± 0.02 , N=12) was higher than in healthy controls (0.14 ± 0.03 , N=13; $p=0.0004$).

We published previously on Fractional Loss 30'–120' in PD [11]. Our data indicating accelerated loss of putamen F-DOPA-derived radioactivity in PD agree with those reported by Sossi and colleagues, who devised and applied a complex kinetic compartmental model [27]. From extending the analysis to other groups, we now report that increased Fractional Loss 30'–120' is found in parkinsonian disorders across a variety of probable pathogenetic mechanisms--neuronal synucleinopathy in PD, glial synucleinopathy in MSA, and tauopathy in PSP.

Although a vesicular storage defect would be expected to accelerate the loss of F-DOPA-derived radioactivity, this does not necessarily mean that the finding of accelerated loss indicates a vesicular storage defect. An alternative explanation is increased release coupled

with decreased reuptake [27]. The current data about Fractional Loss, considered in isolation, is consistent with but does not necessarily imply decreased vesicular storage.

Post-Mortem Putamen Dopaminergic Innervation

Denervation—The patient groups with MSA, Gaucher/PD, or sporadic PD had markedly decreased putamen DA concentrations (Figure 5). Mean tissue DOPA concentrations were variably decreased (26% of control in MSA, p =non-significant; 35% of control in Gaucher/PD, p =0.12; 38% of control in sporadic PD, p =0.012).

Vesicular Storage—Of four conditions with putamen dopaminergic denervation in which post-mortem neurochemical data were available, all had decreased DA:DOPA ratios. As expected from drastically decreased DA but only modestly decreased tissue DOPA concentrations, putamen DA:DOPA ratios were decreased to a smaller extent than was DA in the MSA, Gaucher/PD, and sporadic PD groups, (33.0% vs. 4.3% of control in MSA, 5.5% vs. 3.7% in Gaucher/PD, and 10.2% vs. 6.0% in sporadic PD; Figure 5). Individual values for putamen DA:DOPA ratios were positively correlated with putamen DA content (Figure 5C).

Others have shown that striatal:occipital cortex ratios are equally effective as k_3 in the 2-compartment kinetic model for distinguishing PD from control subjects, and in the revision we cite two of these reports, by Jokinen et al. (J Nucl Med 2009; 50:893–899) and by Dhawan et al. (J Nucl Med 2002; 43:1324–1330). Our data about PD agree fully with those reported previously.

In Vivo Myocardium Noradrenergic Innervation

Denervation—As shown in Figures 6 and 7, several patient groups with diverse conditions had cardiac noradrenergic denervation, identified by low values for ^{18}F -DA-derived radioactivity at 8' after initiation of 3-minute infusion of the tracer (8' Radioactivity).

Vesicular Storage—Of 10 conditions with cardiac sympathetic denervation indicated by low ^{18}F -DA-derived radioactivity, 9 had accelerated loss of the radioactivity (Figure 7). The sole exception was SNS-x, in which one would not expect a neurodegenerative process in the residual neurons. The rate of loss of ^{18}F -DA-derived radioactivity was normal in all of five control groups (AAG, MSA-C, MSA-P, POTS, Normal Volunteers). The MSA-C and MSA-P patients had normal mean 8' Radioactivity and $k_{18'-25'}$, whereas the MSA-D patient with low 8' Radioactivity had high $k_{18'-25'}$.

Post-Mortem Myocardium Noradrenergic Innervation

Denervation—As shown in Figure 8, most PD patients and patients with PAF or MSA+D had markedly decreased myocardial NE concentrations. Gaucher/PD patients had moderately decreased NE. Two MSA-P patients had normal NE (clinical characterization of another MSA patient was not supplied).

Vesicular Storage—Among 3 conditions with cardiac sympathetic denervation for which post-mortem neurochemical data were available, all involved increased myocardial

DHPG:NE ratios (Figure 8). Across individual patients with PD, PAF, or MSA, tissue DHPG:NE ratios were negatively correlated with myocardial NE content. The MSA-D patient with low post-mortem myocardial NE content and an elevated DHPG:NE ratio had low in vivo 8' Radioactivity and high k18'–25'.

REGIONAL DENERVATION AND DECREASED VESICULAR STORAGE

Certain patient groups had putamen dopaminergic denervation without cardiac noradrenergic denervation (MSA-P), putamen dopaminergic denervation with generalized sympathetic denervation (PD+OH), generalized sympathetic denervation without putamen dopaminergic denervation (PAF), dopaminergic denervation with sympathetic denervation predominantly in the heart (PD No OH), decreased cardiac noradrenergic innervation without a neurodegenerative disease (SNS-x), or decreased post-ganglionic sympathetic outflows without cardiac noradrenergic denervation (AAG). In all these groups, evidence for deficient vesicular storage was obtained only in conditions and regions with degenerative catecholaminergic denervation.

In PAF, results of ^{18}F -DOPA and ^{18}F -DA scanning indicated intact putamen dopaminergic innervation and myocardial noradrenergic denervation, whereas in MSA the scanning results indicated generally decreased putamen dopaminergic innervation and intact myocardial noradrenergic innervation. In both groups, evidence for a vesicular storage defect was found only in the denervated region (increased putamen k30–120' for ^{18}F -DOPA, increased septal k8'–25' for ^{18}F -DA). Post-mortem, decreased DA:DOPA ratios were found in MSA (Figure 5B) but not in PAF (59.6% of control, N=3, p=0.78), and increased DHPG:NE ratios were found in PAF and MSA+D but not in MSA-P (Figure 9).

Patient groups with PAF or PD+OH had high values for peak arterial plasma F-DOPAC:DHPG (Figure 9), whereas groups without generalized noradrenergic denervation did not. In particular, the PD No OH group had normal mean peak F-DOPAC:DHPG (Figure 9, pink arrow). Across groups, peak F-DOPAC:DHPG was negatively related with arterial plasma DHPG (Figure 9, Insert). Therefore, in vivo evidence for decreased vesicular uptake in sympathetic noradrenergic neurons in the body as a whole is found only in diseases known to involve degenerative loss of sympathetic noradrenergic neurons and not in diseases involving dysfunction of intact neurons (e.g., MSA-P, MSA-C, AAG) or denervation localized to the heart (PD No OH, SNS-x).

DECREASED VESICULAR STORAGE ACROSS ETIOLOGIES AND PATHOGENETIC MECHANISMS

Data from patient groups with inherited neurodegeneration confirmed an association between catecholaminergic denervation and a vesicular storage defect across disease etiologies—PARK1 from mutation of the gene encoding alpha-synuclein, PARK4 from triplication of the alpha-synuclein gene, PARK8 from mutation of the LRRK2 gene, FD from mutation of the IKBKAP gene, and Gaucher/PD from mutation of the gene encoding glucocerebrosidase. Evidence for such an association was also obtained in diseases involving likely different pathogenetic mechanisms, since the rates of loss of putamen ^{18}F -

DOPA-derived radioactivity were increased in PSP (tauopathy), PD (intra-neuronal synucleinopathy), and MSA-P (glial synucleinopathy).

Although most MSA patients had neuroimaging evidence for intact cardiac sympathetic innervation and normal loss of ^{18}F -DA-derived radioactivity, an MSA-P patient with autopsy proven cardiac sympathetic denervation and no intra-neuronal alpha-synuclein deposition had accelerated loss of ^{18}F -DA-derived radioactivity, indicating a link between cardiac noradrenergic denervation and a vesicular storage defect in the residual nerves even in MSA without neuronal alpha-synucleinopathy. Two other patients, who had PSP and therefore presumably a tauopathy, also had both low ^{18}F -DA-derived radioactivity and accelerated loss of the radioactivity. Thus, in Lewy body and as well as in non-Lewy body diseases, the in vivo data about the rate of loss of ^{18}F -DA-derived radioactivity point to an association between cardiac noradrenergic denervation and a vesicular storage defect.

PERSPECTIVE

Several studies have noted decreased binding of tetrabenazine or hydrotetrabenazine in PD putamen [22, 28–30], demonstrating decreased availability of the type 2 VMAT; however, this approach cannot easily separate denervation from decreased VMAT2 expression or decreased vesicle populations in residual neurons as determinants of decreased VMAT2 availability. The same limitation applies to decreased cardiac ^{123}I -metaiodobenzylguanidine-derived radioactivity in a variety of neurocardiologic disorders [31, 32].

A recent study provided direct post-mortem evidence for decreased uptake by vesicles isolated from putamen tissue in PD [33]. Both decreased tetrabenazine binding and decreased ^3H -DA uptake per VMAT2 binding site were noted, suggesting that decreased vesicular storage may reflect both decreased VMAT2 availability and function. Thus, decreased vesicular storage could be due to multiple factors, such as decreased availability of ATP required for the energy-requiring vesicular uptake process, decreased expression or activity of VMAT2 [34, 35], or decreased axonal transport of vesicles or vesicle-associated proteins [36–38].

IMPLICATIONS FOR THE PATHOGENESIS OF PD AND RELATED DISORDERS

Since DA is synthesized in the neuronal cytoplasm, a vesicular storage defect in putamen terminals could promote accumulation of cytoplasmic DA and consequently increase formation of cytotoxic products of DA metabolism [33, 39–41]. There are two general mechanisms by which buildup of cytoplasmic catecholamine may contribute to the death of catecholamine neurons. First, catecholamines such as DA oxidize spontaneously to quinones, chromes, and indoles, each of which may be toxic. DA-Quinone interferes with mitochondrial functions [42] including complex I [43], inhibits proteasomal function [44], and reacts with sulfur residues in glutathione to form cysteinyl-DA or with cysteine residues in proteins, altering protein functions [45]. Second, cytoplasmic DA is oxidized enzymatically by monoamine oxidase (MAO) to produce hydrogen peroxide and 3,4-

We hope that future studies will explore further and in a more concerted and complete fashion the concept introduced here of a vesicular storage defect as a common, pathogenetically significant characteristic of dying catecholamine neurons.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AAG	autoimmune autonomic ganglionopathy
ALDH	aldehyde dehydrogenase
AMYL	amyloidosis
CAF	chronic autonomic failure
DA	dopamine
DHPG	3,4-dihydroxyphenylglycol
DOPAL	3,4-dihydroxyphenylacetaldehyde
DOPAC	3,4-dihydroxyphenylacetic acid
FD	familial dysautonomia
LRRK2	leucine-rich repeat kinase 2
MAO	monoamine oxidase
MSA	multiple system atrophy
NE	norepinephrine
OCC	occipital cortex
PAF	pure autonomic failure
PD	Parkinson's disease
PET	positron emission tomography
POTS	postural tachycardia syndrome
PSP	progressive supranuclear palsy
PUT	putamen
SNS-x	status post bilateral thoracic sympathectomies
VMAT	vesicular monoamine transporter

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APPENDIX I: METHODS TO ASSESS VESICULAR STORAGE IN CATECHOLAMINERGIC NEURONS

Putamen Dopaminergic Innervation and Vesicular Storage

In vivo

After i.v. administration of ^{18}F -DOPA, peak putamen ^{18}F -DOPA-derived radioactivity is attained at about 30 minutes after i.v. injection of the tracer. Subsequently, putamen radioactivity normally declines slowly over a few hours (Figure 2A). The rate of decline can be quantified from the fractional decrease in radioactivity between the mid-point of the 15' scan beginning at 30' and the mid-point of the 15'-minute scan ending at 120' (Fractional Loss 30'-120'). Denervation alone would be expected to shift downward the curve relating putamen radioactivity vs. time, without a change in the slope (blue arrow in Figure 2B). A vesicular storage defect in the residual terminals would increase the rate of decline in ^{18}F -DOPA-derived radioactivity (Figure 2C).

For purposes of data analysis, putamen radioactivity usually is adjusted for radioactivity in a reference region with scarce dopaminergic innervation, such as the occipital cortex. A straightforward, kinetic model-independent approach uses the putamen:occipital cortex (PUT:OCC) ratio of ^{18}F -DOPA-derived radioactivity [1–3]. Radioactivity concentrations in the putamen (PUT) and occipital cortex (OCC) regions of interest were converted to units of nCi-kg/cc-mCi, reflecting the radioactivity concentration (nCi/cc) at a given dose (in mCi) per kg body mass. Hypertension increases peak PUT ^{18}F -DOPA-derived radioactivity but does not affect PUT:OCC ratios [4]. Therefore, the ratio of PUT:OCC radioactivity at the midpoint of the 15-minute static image beginning at 30' (mean about 38'), called PUT:OCC 30', was taken as a measure of putamen dopaminergic innervation. The fractional loss of PUT radioactivity was measured between the midpoint of the 15-minute static image beginning at 30' (mean about 38') and the midpoint of the last 15-minute static image ending at 120' (mean about 113'). The fractional loss was called Fx Loss 30'-120'. An association of putamen dopaminergic denervation with a vesicular storage defect in the residual terminals would be reflected by an inverse relationship between the Fractional Loss 30'-120' and PUT:OCC 30' across diagnostic groups. To assess whether there is an association between nigrostriatal dopaminergic denervation and a vesicular storage defect in putamen DA terminals, across a variety of diseases, we conducted brain ^{18}F -DOPA PET scanning in patients with different conditions involving parkinsonism—PD+OH, PD No OH, MSA-P, and PSP—or not involving parkinsonism—MSA-C, PAF, AAG, SNS-x, r/o CAF, and healthy volunteers.

Post-mortem

By reference to Figure 1, since the putamen tissue content of DA is for all practical purpose in the vesicles, while DA is produced in the cytoplasm from DOPA, the immediate product of the rate-limiting enzyme in DA synthesis (tyrosine hydroxylase, TH), a vesicular storage defect in putamen dopaminergic terminals would be expected to result in a proportionately greater decrease in the tissue content of DA than of DOPA and therefore in a decreased DA:DOPA ratio [5]. An association of putamen dopaminergic denervation with a vesicular storage defect in the residual terminals would be reflected by an inverse relationship between putamen DA:DOPA ratios and DA contents across diagnostic groups. We predicted that in sporadic PD, Gaucher/PD, and MSA-P, which involve likely different pathogenetic mechanisms of putamen DA depletion, the putamen tissue content of DA would be low with respect to that of DOPA, because of deficient vesicular storage in the residual terminals.

Sympathetic Noradrenergic Innervation and Vesicular Storage

To assess whether loss of sympathetic noradrenergic nerves is associated with deficient vesicular storage regardless of the specific disease, we analyzed in vivo and post-mortem data about indices of vesicular storage from patients with diseases involving or not involving cardiac noradrenergic denervation.

In vivo

Cardiac noradrenergic denervation—If a vesicular storage defect characterized degenerating cardiac sympathetic neurons, then after i.v. injection of the sympathetic neuroimaging agent, ^{18}F -DA, patients with cardiac sympathetic denervation would have accelerated loss of the radioactivity, in a manner analogous to that for ^{18}F -DOPA in the putamen. By reference to Figure 2D, peak ^{18}F -DA-derived radioactivity normally is attained at about 8 minutes after initiation of the 3-minute i.v. infusion of the tracer. The radioactivity at 8 minutes (8' Radioactivity) can be used as a measure of neuronal uptake and thereby of innervation. Subsequently the concentration of ^{18}F -DA-derived radioactivity decreases slowly. The slope of the mono-exponential decline between 8' and 25', referred to as $k_{8'-25'}$, can be taken as a measure of this loss. We showed previously that ^{18}F -DA taken up into the neuronal cytoplasm is translocated extremely rapidly into vesicles, which exist in two pharmacokinetic pools [6]. The loss of radioactivity in this interval mainly reflects leakage from the vesicles into the cytoplasm, escape of vesicular reuptake via the vesicular monoamine transporter (VMAT), and formation of the deaminated metabolite, F-3,4-dihydroxyphenylacetic acid (F-DOPAC) [7–9]. Because of decreased neuronal uptake, denervation would be expected to shift downward the curve relating the log of interventricular septal myocardial ^{18}F -DA-derived radioactivity vs. time, without a change in the slope of the mono-exponential decline. That is, 8' Radioactivity would be decreased, without a change in $k_{8'-25'}$. On the other hand, denervation combined with decreased vesicular uptake would be expected to both decrease 8' Radioactivity and increase $k_{8'-25'}$. An association between a vesicular storage defect and cardiac sympathetic denervation would be reflected by an inverse relationship between $k_{8'-25'}$ and mean 8' Radioactivity across patient groups.

Generalized noradrenergic denervation—After neuronal uptake of F-DA, cytoplasmic F-DA is mainly taken up into vesicles, but a minority is deaminated to form F-DOPAC. If there were isolated denervation without a vesicular storage defect, then peak arterial plasma F-DOPAC would be decreased [7]. If there were an isolated defect in vesicular storage without denervation, then peak arterial plasma F-DOPAC would be increased. That is, the effect of one abnormality could offset the effect of the other abnormality. To detect a vesicular storage defect in the setting of denervation, one can adjust F-DOPAC for plasma 3,4-dihydroxyphenylglycol (DHPG), which reflects turnover of NE stores in sympathetic nerves and therefore overall sympathetic innervation [10]. We used peak arterial F-DOPAC:DHPG as a biomarker of decreased vesicular sequestration in the sympathetic nervous system as whole. We predicted that in PAF and PD+OH, peak arterial F-DOPAC:DHPG would be increased [11], whereas in PD No OH, MSA-P, status post SNS-x, or AAG, peak arterial F-DOPAC:DHPG would be normal.

If generalized loss of sympathetic noradrenergic nerves were associated with a vesicular storage defect, regardless of the specific disease, then across conditions peak F-DOPAC:DHPG in arterial plasma would be related inversely to arterial plasma DHPG.

In PD without orthostatic hypotension (PD No OH), noradrenergic denervation seems to be localized to the heart, whereas PD+OH and PAF involve generalized noradrenergic denervation. We therefore predicted normal peak arterial plasma F-DOPAC:DHPG in PD No OH.

Post-mortem

If cardiac sympathetic denervation were associated with a vesicular storage defect, then across patient groups with different diseases, in the groups with myocardial NE depletion the pattern of post-mortem catechols in the left ventricular myocardium would indicate a vesicular sequestration-to-oxidative deamination shift in residual noradrenergic nerves. An elevated ratio of DHPG:NE provides an index of this shift [5]. We measured apical myocardial DHPG:NE ratios in PD, PAF, and MSA with myocardial NE depletion (MSA+D). Although there is no evidence for a vesicular sequestration-to-oxidative deamination shift in MSA overall, we anticipated evidence for such a shift in MSA+D.

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APPENDIX: Detailed Description of Diagnostic Groups

Amyloidosis (AMYL)

AMYL can be associated with neurogenic OH from NE deficiency [1], and, at least in familial AMYL, neuroimaging evidence of cardiac sympathetic denervation [2]. There is insignificant amyloid deposition in the center nervous system [3]. ¹⁸F-DA data were analyzed from 2 AMYL patients with neurogenic OH, both white men with sporadic disease, ages 65 and 66 years old.

Autoimmune Autonomic Ganglionopathy (AAG)

AAG is a rare form of pandysautonomia in which the patients have a circulating antibody to the neuronal nicotinic receptor [4]. Whether the disease involves nigrostriatal dopaminergic denervation is unknown. Based on ¹⁸F-DA PET scanning, cardiac sympathetic innervation is intact in AAG [4, 5]. ¹⁸F-DA data were analyzed from 4 AAG patients (mean age 65 ± 10 years, 2 men and 2 women, all white). ¹⁸F-DOPA data were reviewed from the same patients. The ¹⁸F-DA data were reported previously [5]; the ¹⁸F-DOPA data have not been reported previously.

Familial Dysautonomia (FD)

FD, also called hereditary sensory and autonomic neuropathy, type III) is a rare disease transmitted as an autosomal recessive trait caused by a splicing mutation of the IKBKAP gene [6]. The disease is thought to be associated with arrested development of sympathetic noradrenergic neurons, with neurodegeneration progressing during adult life [7]. We found no literature about the status of putamen dopaminergic innervation in FD. Results of cardiac

sympathetic neuroimaging have indicated partial cardiac sympathetic denervation in FD [8]. ^{18}F -DA data were analyzed from 6 previously reported FD patients (mean age 33 ± 4 years, 2 men, 4 women, all white and of Ashkenazic extraction).

Gaucher/PD

Mutation of the gene encoding glucocerebrosidase causes Gaucher disease, which is transmitted as an autosomal recessive trait. Gaucher disease patients and heterozygous carriers of the mutant gene have an increased risk of PD [9]. Gaucher/PD patients have striatal dopaminergic neuroimaging findings that resemble those in sporadic PD [10]. Based on results of ^{123}I -metaiodobenzylguanidine scanning, Gaucher/PD involves cardiac sympathetic denervation that may be diffuse or localized to the inferoposterior myocardium [11, 12]. We analyzed post-mortem data about putamen catechols in 4 Gaucher/PD patients (mean age 62 ± 4 years, 3 men and 3 women, all white). Putamen and apical myocardial catechols data were reviewed from 2 other Gaucher patients, 1 with Gaucher disease and dementia with Lewy bodies (61 year old white female) and 1 with Gaucher disease and subtle parkinsonism (73 year old white woman). None of the data about Gaucher/PD patients have been published previously.

Healthy Volunteers

^{18}F -DA data were analyzed from 48 healthy volunteers (mean age 48 ± 2 years, 39 men, 11 women, 39 white, 5 African-American) studied at the NIH Clinical Center. Of these, 13 also had ^{18}F -DOPA data. In addition, 10 normal volunteers were pre-treated with desipramine (DMI, 125 mg by mouth about 3 hours before ^{18}F -DA administration), 8 were treated with i.v. trimethaphan (TRI) to block ganglionic neurotransmission, and 2 were treated with i.v. nitroprusside (NIP) to decrease blood pressure and increase sympathetic noradrenergic outflows reflexively. The results of the pharmacologic manipulations have been published previously [13–15].

MSA-P

MSA is classified into parkinsonian and cerebellar subtypes (MSA-P and MSA-C) [16]. In this study MSA patients were classified as having MSA-P if they had parkinsonism, regardless of the presence of cerebellar signs. MSA involves putamen dopaminergic denervation, assessed both in vivo by ^{18}F -DOPA PET scanning [17–21] and post-mortem by neurochemistry [22, 23]. Most MSA patients have intact cardiac and extra-cardiac sympathetic innervation [20, 24], although there are exceptions [25–28]. We analyzed cardiac ^{18}F -DA data from a total of 60 MSA-P patients (mean age 61 ± 1 years, 37 men and 23 women, 49 white, 4 Hispanic, 2 African-American, 2 Asian-American, 1 Greek, 1 Middle Eastern, 1 Indian). There were 27 MSA-P patients who also had putamen ^{18}F -DOPA data. Among the MSA-P patients, 1 did not receive ^{18}F -DA but did receive ^{18}F -DOPA, 1 had no radioactivity attenuation correction and therefore no quantitative ^{18}F -DA data. Another MSA-P patient, who had autopsy proven cardiac sympathetic denervation, is considered separately below. Most of the ^{18}F -DA and ^{18}F -DOPA data in the MSA patients have been published, but with the data lumped across the two subtypes [20, 29].

Post-mortem myocardial and putamen contents of catechols were lumped across the MSA-P and MSA-C groups. Myocardial data were analyzed for 3 and putamen data for 13 MSA patients (Another MSA patient is discussed separately below). Most of the putamen tissue was from the University of Miami Brain Endowment Bank, and the patients had not been studied at the NIH during life. The myocardial data have not been published; the putamen data have recently been accepted for publication [23].

MSA-C

We analyzed cardiac ^{18}F -DA data from a total of 15 MSA-C patients (mean age 57 ± 2 years, 10 men and 5 women, 13 white, 1 Hispanic, 1 African-American). All the MSA-C patients also had putamen ^{18}F -DOPA data. Most of the ^{18}F -DA and ^{18}F -DOPA data in the MSA patients have been published, but with the data lumped across the two subtypes [20, 29].

MSA with Diffuse Cardiac Noradrenergic Denervation (MSA+D)

Although most MSA patients have intact cardiac and extra-cardiac noradrenergic innervation, we reported a case of MSA-P with cardiac sympathetic denervation and no Lewy bodies, implying that cardiac sympathetic denervation can occur without intra-neuronal synucleinopathy [27]. Data from this previously published case are included in this report.

PARK1

PARK1 is a rare form of familial PD in which the disease is transmitted as an autosomal dominant trait [30]. PD in PARK1 results from A53T mutation of the gene encoding alpha-synuclein. PARK1 patients have post-mortem neuropathologic findings typical of sporadic PD [31]. PARK1 is associated with autonomic failure and neuroimaging evidence of cardiac sympathetic denervation [32]. We analyzed ^{18}F -DA PET data from a PARK1 patient, a 48 year old Italian-American man. The patient was the subject of a case report that described an etiologic link between sympathetic neurocirculatory failure and alpha-synucleinopathy in PD [32].

PARK4

PARK4 is a form of familial PD due to triplication of the gene encoding alpha-synuclein [33]. PARK4 patients have neuroimaging evidence of cardiac sympathetic denervation [34]. Although one would presume PARK4 involves in vivo neuroimaging and postmortem neuropathologic features typical of sporadic PD, we were not able to find reports about putamen ^{18}F -DOPA-derived radioactivity or autopsy findings in PARK4. We analyzed ^{18}F -DA PET data from 2 PARK4 patients, a 47 year old white woman with PD+OH and a 41 year old white woman with PD No OH. Data about $k_{18'-25'}$ radioactivity were published previously for this patient [15]. Data about $k_{18'-25'}$ radioactivity in the PARK4 patient with PD No OH have not been reported previously.

PARK 8: (LRRK2/PD)

The PARK8 form of inherited PD is caused by mutation of the gene encoding leucine-rich repeat kinase 2 (LRRK2) [35]. We previously reported a case of PARK8 in which there was decreased putamen ^{18}F -DOPA-derived radioactivity and severely decreased myocardial ^{18}F -DA-derived radioactivity [36]. We analyzed ^{18}F -DA and ^{18}F -DOPA data from this patient.

Parkinson Disease with Orthostatic Hypotension (PD+OH)

A substantial minority of patients with PD have orthostatic hypotension (OH) [37]. PD+OH is distinguished from PD without OH (PD No OH) by neuropharmacologic, neuroimaging, and neurochemical evidence of generalized noradrenergic denervation [38]. All patients with PD+OH have neuroimaging evidence of loss of sympathetic innervation throughout the left ventricular myocardium [39]. We analyzed cardiac ^{18}F -DA PET data from a total of 44 PD+OH patients (including the PARK1 and PARK4 patients who had OH). The PD+OH group had a mean age of 69 ± 1 years, 30 men and 14 women, 41 white and 3 African-American. We reviewed putamen ^{18}F -DOPA data from 26 of these PD+OH patients. Most of the septal ^{18}F -DA and putamen ^{18}F -DOPA data in the PD+OH patients have been published previously [20, 40, 41]. Post-mortem neurochemical data were obtained in 2 PD+OH patients, and the data were lumped with those from other PD patients.

PD No OH

PD is well known to entail severe putamen dopamine depletion [42–44], related to striatal dopaminergic denervation [45, 46] and a vesicular storage defect in the residual terminals [47, 48]. Of PD patients who do not have OH (PD No OH), about one-half have diffuse cardiac noradrenergic denervation by sympathetic neuroimaging. Of the remaining half, most have partial loss of sympathetic innervation confined to the left ventricular free wall or apex [49], and a minority have normal innervation. PD patients have profoundly decreased epicardial tyrosine hydroxylase immunostaining, indicating cardiac sympathetic denervation [25, 26, 46, 50–54]. ^{18}F -DA data were analyzed from a total of 48 PD No OH patients (mean age 59 ± 1 years, 37 men and 11 women, 43 white, 2 African-American, 1 Middle Eastern). Of the PD No OH patients, 1 had PARK4, 1 LRRK2 mutation, and 1 Gaucher/PD. The Middle Eastern patient with PD No OH was diagnosed subsequently with progressive supranuclear palsy (PSP). A total of 26 PD No OH patients had putamen ^{18}F -DOPA PET scanning.

Postural Tachycardia Syndrome (POTS)

POTS is a relatively common, poorly understood condition, mainly in adult women, that involves excessive tachycardia responses to orthostasis, accompanied by a variety of non-specific and often debilitating symptoms such chronic fatigue, exercise intolerance, decreased ability to concentrate (“brain fog”), gastrointestinal problems, and chronic pain. Pathogenetic mechanisms of POTS are likely to be heterogeneous and may include patchy sympathetic denervation [55], abnormal regulation of extracellular fluid volume [56], decreased neuronal uptake mediated by the cell membrane NE transporter [57]. We found

no literature about the status of putamen dopaminergic innervation in POTS. Myocardial ^{18}F -DA-derived radioactivity is generally normal in POTS [58]. Data about retention of ^{18}F -DA-derived radioactivity in POTS patients have not previously been reported. ^{18}F -DA data were analyzed from 16 POTS patients (mean age 41 ± 3 , 1 man and 15 women, all white).

Progressive Supranuclear Palsy (PSP)

PSP is thought to reflect a tauopathy, rather than an intra-neuronal synucleinopathy as in PD or glial synucleinopathy as in MSA [59, 60]. The disease is characterized by a vertical gaze palsy. PSP entails in vivo and post-mortem neurochemical evidence of striatal dopaminergic denervation [61]. Patients often carry a diagnosis of PD for years before the diagnostic ophthalmologic sign emerges. This was the case in 1 of the patients included in this report. All the neuroimaging and neurochemical data from the PSP patients have not been published previously.

Pure Autonomic Failure (PAF)

PAF is a rare Lewy body disease [62] in which the patients have neurogenic OH and generalized noradrenergic denervation without clinical evidence of central neurodegeneration. PAF does involve central DA deficiency but without neuroimaging evidence of loss of dopaminergic innervation [63]. ^{18}F -DA data were analyzed from 17 PAF patients (mean age 67 ± 3 years, 11 men and 6 women, all white); in 1 patient the PET data were not interpretable. ^{18}F -DOPA data were reviewed for 16 PAF patients, CSF DOPAC from 23, and peak plasma F-DOPAC:DHPG from 11. Most of the ^{18}F -DA and ^{18}F -DOPA data from PAF patients were published previously [15, 29, 63]. Post-mortem myocardial and putamen catechols data were obtained from 3 PAF patients. The post-mortem myocardial and putamen catechols data in this group have not been published previously.

r/o CAF

^{18}F -DOPA data were analyzed from patients referred for dysautonomia who had no evidence of chronic autonomic failure, parkinsonism, or cerebellar ataxia (r/o CAF, N=11, mean age 53 ± 3 years, 7 men and 4 women, all white).

Thoracic Endoscopic Sympathectomies (SNS-x)

Data were analyzed from a group of patients who had undergone bilateral thoracic endoscopic sympathectomies, usually for hyperhidrosis. There is no published evidence about the status of putamen dopaminergic innervation in SNS-x. Such patients have partial loss of cardiac sympathetic innervation distributed about equally in portions of the left ventricular myocardium [64]. ^{18}F -DA and ^{18}F -DOPA data were reviewed from 5 SNS-x patients (mean age 38 ± 3 years, 4 men and 1 woman, 3 white, 1 Middle Eastern, 1 Indian). Data from the present cohort of SNS-x patients have not been previously reported.

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HIGHLIGHTS

We review here evidence that a variety of clinical conditions involving degenerative loss of catecholamine neurons have in common a vesicular storage defect in the residual neurons. This abnormality seems to characterize dying catecholamine neurons regardless of the specific disease etiology, pathogenetic mechanisms, or lesion locations.

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Figure 1. Concept diagram depicting synthesis and intra-neuronal metabolic fate of dopamine (DA)

A vesicular storage defect would be expected to decrease vesicular DA content (DA) and the ratio of DA:DOPA. Decreased vesicular sequestration would shift the fate of cytoplasmic DA toward auto-oxidation to form quinones and toward enzymatic oxidative deamination catalyzed by monoamine oxidase-A (MAO-A) to form the catecholaldehyde, 3,4-dihydroxyphenylacetaldehyde (DOPAL). DA-quinone reacts with glutathione or cysteine to form 5-S-cysteinyl-DA and bonds covalently with cysteine residues in proteins. DOPAL auto-oxidizes to DOPAL-quinone, generates reactive oxygen species, bonds covalently with amine groups on lysine residues in proteins, and oligomerizes alpha-synuclein. Decreased aldehyde dehydrogenase (ALDH) attenuates metabolic conversion of DOPAL to 3,4-dihydroxyphenylacetic acid (DOPAC).

Figure 2. Diagrams depicting definitions of Fractional Loss 30'–120' from ^{18}F -DOPA PET data and 8' Radioactivity and $k_{8'-25'}$ from ^{18}F -dopamine PET data

As a measure of loss of ^{18}F -DOPA-derived radioactivity after vesicular loading, the fractional loss in radioactivity (Fractional Loss 30'–120') was calculated from the radioactivity in the scan beginning at 30' and the scan ending at 120'. Denervation alone (or blockade of the cell membrane DA transporter) would be expected to decrease peak ^{18}F -DOPA-derived radioactivity (blue arrow). Denervation with decreased vesicular storage would be expected to both decrease peak PUT radioactivity and increase Fractional Loss 30'–120'. As a measure of neuronal uptake and therefore of innervation density in the myocardial interventricular septum, 8' Radioactivity was measured at the midpoint of the 5-minute frame beginning at about 3.5 minutes after initiation of the 3-minute infusion of ^{18}F -dopamine. As a measure of loss of ^{18}F -dopamine-derived radioactivity, the slope of mono-exponential decline in radioactivity ($k_{18'-25'}$) was calculated from radioactivity at 8', 13', 18', and 25'. Previous work has shown that the decline is often bi-exponential, reflecting different rate constants for loss from 2 intra-neuronal vesicular pools. Denervation with decreased vesicular storage would be expected to decrease 8' Radioactivity and increase $k_{18'-25'}$.

Figure 3. Mean ($\pm$ SEM) values for (left) the fractional loss of ^{18}F -DOPA-derived radioactivity between 30 and 120 minutes (Fractional Loss 30'–120') and (right) mean CSF DOPAC as a function of Fractional Loss 30'–120' in parkinsonian and non-parkinsonian conditions Patients with parkinsonism had elevated values for the fractional loss of radioactivity, whereas patients without parkinsonism had normal values; CSF DOPAC was low in conditions involving parkinsonism.

Figure 4. Mean ($\pm$ SEM) values for putamen ^{18}F -DOPA-derived radioactivity as a function of time after i.v. administration of ^{18}F -DOPA in patient groups with (red arrows) or without (green arrows) parkinsonism

Gray circles indicate normal mean values. Parkinsonian patients had accelerated loss of putamen ^{18}F -DOPA-derived radioactivity; non-parkinsonian patients did not.

Figure 5. Dopamine content and an index of vesicular storage in putamen of patients with parkinsonism and controls

(A) Mean (±SEM) values for putamen tissue DA in MSA (blue), Gaucher/PD (yellow), sporadic PD (red), and controls (gray). Numbers in italics are p values for comparisons with control subjects, based on post-hoc testing after ANOVA. (B) Mean (±SEM) values for DA:DOPA. (C) Individual data for DA:DOPA as a function of DA content. Patient groups with different disease etiologies (sporadic PD, Gaucher PD, MSA) had in common DA depletion and decreased vesicular storage. Across individuals, DA:DOPA was positively related with DA.

Figure 6. Myocardial interventricular septal ^{18}F -DA-derived radioactivity (means $\pm$ SEM) as a function of time in patient groups with (red arrows) and without (green arrows) sympathetic denervation

Abbreviations: PARK1=familial Parkinson's disease (PD) from A53T mutation of the gene encoding alpha-synuclein; PARK4=familial PD from alpha-synuclein gene triplication; Amyloid=acquired amyloidosis; PAF=pure autonomic failure; PD+OH=PD with orthostatic hypotension; FD=familial dysautonomia; MSA=multiple system atrophy;

AAG=autoimmune autonomic ganglionopathy. Also shown are data for normal volunteers treated with 125 mg of oral desipramine (DMI) before ^{18}F -dopamine administration.

Conditions involving sympathetic denervation are associated with accelerated declines of septal ^{18}F -DA-derived radioactivity.

Figure 7. Scatter plot relating mean values for k to mean values for $8'$ ¹⁸F-DA-derived radioactivity in various patient groups

Pink rectangle indicates groups with diffuse cardiac sympathetic denervation, green partial cardiac sympathetic denervation, and blue intact cardiac sympathetic innervation. All patient groups with septal sympathetic denervation as indicated by low $8'$ ¹⁸F-DA-derived radioactivity had accelerated loss of radioactivity. Additional abbreviation:

AMYL=amyloidosis; MSA-P=parkinsonian form of MSA; MSA-C=cerebellar form of MSA; MSA+D=MSA with cardiac sympathetic denervation, defined by $8'$ radioactivity more than 2 standard deviations below the normal mean; NL=normal; POTS=postural tachycardia syndrome; PSP=progressive supranuclear palsy;.

Figure 8. Scatter plot relating individual values for the ratio of DHPG:NE as a function of NE content in apical myocardial tissue

Additional abbreviation: DLB=dementia with Lewy bodies. Note that regardless of diagnosis, DHPG:NE, an index of a vesicular sequestration-to-oxidative deamination shift in the fate of cytoplasmic NE, is associated with myocardial NE depletion.

Figure 9. Mean values ($\pm$ SEM) for peak arterial plasma FDOPAC:DHPG in various patient groups

Additional abbreviations: PD No OH=PD without orthostatic hypotension. Note elevated peak FDOPAC:DHPG in PD+OH, which is associated with generalized noradrenergic denervation, and normal peak FDOPAC:DHPG in PD No OH, which is associated with relatively selective cardiac noradrenergic denervation.

Table 1

Condition	Gp. #	DA Den.	Ex Loss 30'-120'	N	PUT DA:DOPA	N
<i>Post-mortem</i>						
AAG	1		NORMAL	4		
Amyloidosis	2	NORMAL	NORMAL	1		
Control	3	NORMAL	NORMAL	11	NORMAL	18
FD	4					
Gaucher/PD	5	ABNORMAL	ABNORMAL	1	ABNORMAL	6
LRRK2	6	ABNORMAL	ABNORMAL	1		
MSA (Combined)	7	ABNORMAL	ABNORMAL		ABNORMAL	8
MSA-C	8	ABNORMAL	ABNORMAL	12		
MSA-P	9	ABNORMAL	ABNORMAL	27		
MSA+D	10	ABNORMAL	ABNORMAL	1	ABNORMAL	1
Healthy Volunteer	11	NORMAL	NORMAL	13		
PAF	12	NORMAL	NORMAL	16	NORMAL	3
PARK1	13					
PARK4	14	ABNORMAL				
PD (Combined)	15	ABNORMAL	ABNORMAL		ABNORMAL	17
PD No OH	16	ABNORMAL	ABNORMAL	25		
PD+OH	17	ABNORMAL	ABNORMAL	23		
POTS	18					
PSP	19	ABNORMAL	ABNORMAL	2		
SNS-x	20		NORMAL	5		
TOTAL 195				142		53

Condition	Gp.	# SNS Denerv.	k8'-25' <i>mir^h-1</i>	N	Plasma FDOPAC:DHPG	N	MYO DHPG:NE	N
<i>Post-mortem</i>								
AAG	1	NORMAL	NORMAL	4				
Amyloidosis	2	ABNORMAL	ABNORMAL	2				
Control	3	NORMAL	NORMAL		NORMAL	2	NORMAL	23
FD	4	ABNORMAL	ABNORMAL	6				
Gaucher/PD	5	ABNORMAL	ABNORMAL	1			ABNORMAL	2

Condition	Gp.	# SNS Denerv.	k8'-25'	N	Plasma	FDOPAC:DHPG	N	MYO DHPG:NE	N
LRRK2	6	ABNORMAL							
MSA (Combined)	7	NORMAL	NORMAL	15			2		
MSA-C	8	NORMAL	NORMAL	59			21	NORMAL	3
MSA-P	9	NORMAL	NORMAL	1				ABNORMAL	1
MSA+D	10	ABNORMAL	ABNORMAL	48			26	NORMAL	23
Healthy Volunteer	11	NORMAL	NORMAL	17			11	ABNORMAL	1
PAF	12	ABNORMAL	ABNORMAL	1					
PARK1	13	ABNORMAL	ABNORMAL	2					
PARK4	14	ABNORMAL	ABNORMAL	46			7	ABNORMAL	11
PD (Combined)	15	ABNORMAL	ABNORMAL	44			12		
PD No OH	16	NORMAL	ABNORMAL	16					
PD+OH	17	ABNORMAL	ABNORMAL	2					
POTS	18	NORMAL	NORMAL	7					
PSP	19	ABNORMAL	ABNORMAL	5					
SNS-x	20	ABNORMAL	NORMAL	271					
TOTAL	421			86					64

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